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FERMENTATION[1]

II.

Études sur la Bière. Par M. L. Pasteur. (Paris: Gauthier-Villars.)

WHAT is fermentation? Such is the important question of which we have now to seek the solution. It is necessarily impossible within the scope of a brief article to enter into an examination of the numerous theories propounded, yet a few words of explanation are needed in order that we may the more clearly understand what it is that Pasteur has done, and what is the real bearing of his answer to the question not only on fermentation, but on many a disease which flesh is heir to.

According to Willis and Stahl, to whom we are indebted for the first philosophical attempt at explanation, fermentation was due to a peculiar motion communicated from a decaying body to another substance, by which a similar degrading action was set up, so that complex molecules became less complex. This idea was subsequently taken up and developed by Gay-Lussac, Liebig, and others, who maintained that the decomposition of albuminous bodies produced the molecular disturbance by which sugar was broken up into alcohol and carbonic acid. Thus the molecular disturbance produced by decay of nitrogenous matter in a fitting liquid gave rise to lactic and butyric acids and other products.

The contact theory of Berzelius and Mitscherlich belonged to a period in the history of chemistry when catalysis was constantly employed to explain the unknown, and need not here be further alluded to.

Cagniard de Latour, in 1837, first proved that yeast is composed of living cells; this was confirmed by the almost contemporaneous observations of Schwann and Kützing, and subsequently by Turpin and others. The subject, however, remained in much obscurity until Pasteur commenced his investigations on the nature of ferments; these have been carried on for a period of twenty years, the results obtained being communicated to the French Academy in 1862 and subsequent years and are now, in so far as they concern the brewer, summed up in the important work before us. The theory of molecular disturbance produced by putrefaction, so energetically maintained by Liebig, may be considered vanquished on all points. M. Pasteur, from his researches, has proved incontestably the two following propositions:—

1. *Every diseased alteration in the quality of beer coincides with the development of microscopic organisms foreign to the nature of beer, properly so called.*
2. *The absence of alteration of beer wort and of beer coincides with the absence of foreign organisms.*

The first proposition M. Pasteur has amply proved from hundreds of examinations of beer undergoing diseased action; in no one case did he find the sediment of such beer to consist solely of the globular or ovoid alcoholic yeast cells, but of a more or less intermixture with the various ferments of disease already described by him with great minuteness in his "Études sur le Vin." His second proposition is a necessary corollary of the first.

We cannot do better than follow our author in some of his researches by which he has demonstrated the truth of these propositions. M. Pasteur, by boiling animal and vegetable infusions—it is not necessary in all cases to use so high a temperature—and then, by cooling them under rigid precautions against the introduction of germs from the air, has shown that they are then unacted upon by oxygen, at least that no putrefaction or fermentation ensues.

The temperature to which a liquid must be heated in order to ensure against subsequent decomposition varies with its nature. Thus while 50° C. is sufficient to destroy the germs in vinegar, malt infusion must be heated to 90° C., and milk to 110° C., and others require even higher temperatures. Blood and urine heated in Pasteur's flasks and cooled under conditions precluding the entrance of germs from the air undergo no alteration even when kept for weeks at a temperature of 20° C. in contact with pure air. Mere oxidation therefore is not the cause of fermentation and putrescence. It is unnecessary to refer to the exceptions taken by many experimentalists to Pasteur's conclusions, the great experimental skill required and the rigid precautions to be taken to secure success amply explain why others less habile than Pasteur have obtained conflicting evidence. Two of the ablest of his opponents, Doctors Brefeld and Traube, have recently retracted their previous objections to Pasteur's results, and now unhesitatingly adopt his views on Fermentation. Allusion has already been made to the fact that the brewer occasionally, and the wine grower invariably, leaves the saccharine liquid to spontaneous fermentation; it is many years since Pasteur first demonstrated that the dust on the skin of the grape contained the small organisms necessary for vinous fermentation, and that when these were destroyed or removed no fermentation took place. Thus the juice heated in a Pasteur flask with the usual precautions against the admission of germ-laden air did not undergo fermentation when freely exposed to germ-free air, but did so readily when a portion of the dust of the skin was added. If this dust, however, was removed by a few drops of water and then boiled previously to being added to the must no fermentation took place. Hence the dust on the skin contained the ferments, and M. Pasteur has demonstrated their nature and their distinguishing features.

In the case of boiling beer wort there are necessarily no living organisms, and therefore, as Pasteur has shown, there is no change when the wort is allowed to cool in a vessel to which only pure air has access. If, however, the cooled wort be exposed to air, ferments of various kinds, *e.g.*, *Saccharomyces cerevisiæ* (though rarely), *Sacchoromyces pastorianus*, and *apiculatus*, and other alcoholic ferments, fall into the liquid; at the same time, however, attended by more or less of the lactic and butyric ferments and other organisms of disease. In a brewery where other fermentations are proceeding, doubtless the number of alcoholic ferments will be greater and the injurious bacteria less than in other localities; still in any case the beer when finished must be very inferior, owing to the large quantities of acetic, lactic, and butyric acids, and other products of putrefactive change. M. Pasteur, having satisfactorily demonstrated that alcoholic fermentation was due to the action of certain organisms living and reproducing in a saccharine liquid, the question arose whether the common *Penicillium glaucus* and the *Aspergillus glaucus* could be transformed into the true alcoholic ferments of beer or of wine, and the still wider question of the conversion of one ferment into another; a question of vital importance to the brewer, since, if this theory of the conversion of one kind of ferment into another were proved to be true, the hope of obtaining greater power over the results of his process would be dashed to the ground, or at any rate such power would only be obtained in the distant future. In his researches upon this question, M. Pasteur availed himself of the liquid proposed by his pupil, M. Raulin (p. 89), *e.g* :—

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[1] Continued from p. 216.

Water	1,500	parts.
Cane sugar	70	,,
Tartaric acid	4	,,
Nitrate of ammonia	4	,,
Phosphate of ammonia ...	0·6	,,

We must refer the reader to M. Pasteur's book for the full directions of procedure and the precautions to be taken in order to avoid the introduction of mixed organisms. No one can read this portion of his work without being greatly impressed by the author's wonderful ability in detecting and overcoming difficulties and thereby removing sources of error which have led so many to erroneous conclusions. We may briefly sum up this part of his work by stating that in no case where such necessary precautions were taken did he ever find that the penicillium changed to alcoholic yeast or to a mycoderma, or that yeast gave rise to vibrios, or that mycoderma aceti became yeast. M. Pasteur himself at one time thought he had detected the conversion of the mycoderma vini into a true alcoholic ferment; in this, however, he now finds he was in error, and that more rigorous precautions fail to support his original supposition.

In his experiments with mycoderma aceti and mycoderma vini carried out with his usual marvellous ingenuity and attention to details, he has shown that these organisms in contact with the air burn up the sugar of the liquid upon which they rest; and so far from alcohol being produced in quantities that may be detected, the mycoderma vini completely burns up any alcohol previously existing in the liquid. When submerged in the liquid, though life is far less active, they continue to exist for a time, undergoing structural changes, obtaining the necessary oxygen from the sugar, and producing alcohol, as in the case of the penicillium glaucus and aspergillus glaucus under similar conditions of submersion. We must admit, with Pasteur, that the production of alcohol and carbonic acid from sugar, in other words, alcoholic fermentation, is a chemical act attending the life of cells the moment they are removed from the influence of free oxygen, and are submerged in a saccharine liquid capable of yielding oxygen and heat by its decomposition. This alcohol-producing character becomes, therefore, not the isolated property of this or that organism, but a general function of a living cell, when removed from the air and compelled to obtain the necessary oxygen by the decomposition of its food. The duration of life and the production of alcohol will vary with the power of the cell to reproduce its kind under these submerged conditions. I have already referred to the production of alcohol and carbonic acid when growing malt is placed in an air-tight vessel. Under such circumstances alcohol and a large amount of carbonic acid are for a long time produced, ultimately the moist mass becomes increasingly acid, the production of alcohol ceasing and acetic and other acids being formed attended with a strong etherial odour. When first noticed by the writer in the year 1873 he was unaware of M. Pasteur's previous conclusions as to the action of vegetable cells. Messrs. Lechartier and Bellamy in 1874 published their valuable researches, upon which they had been engaged for some time, and they proved incontestably that the cells of fruits possessed the power, when removed from free oxygen, of obtaining this gas by the decomposition of sugar; alcohol and carbonic acid gas being produced, though not in the ratio found in true alcoholic fermentation. We thus learn from our author's researches, amply confirmed by those of Lechartier and Bellamy, that active living cells, when removed from the influence of free oxygen, and when placed under conditions where they must obtain it from saccharine materials, for a time continue to exist, producing alcohol and carbonic acid. Those, such as the cells of fruits and grain, which cannot reproduce under these conditions, soon die, and the amount of alcohol produced is but small; on the other hand those cells which can reproduce by germination become true ferments, and their action only ceases when the original reproductive energy due to their previous aërial condition also ceases. Thus when the *mucor racemosus* was submerged in a saccharine liquid it was found that the alcoholic fermentation was at first active and then finally ceased, the cells being deformed in contour, full of granulations, and to all appearance dead. The introduction of a little pure air, however, reinvigorated the apparently dead organism, and again—for a time—alcoholic fermentation was produced. Free oxygen therefore endows these simple cell-plants with an energy which enables them for a time, longer or shorter, to exist without it by obtaining the necessary oxygen from their food. I have said *for a time*, because the mucor racemosus, or the mycoderma submerged in a liquid soon lose their activity and cease to act, the true yeast ferments under the same conditions being enabled for a much longer period to reproduce and carry on their vital functions; even with these, however, Pasteur has proved the necessity of periodical aëration so as again to place them under their normal aërial condition, a fact long known to those English brewers who employ the method of *rousing* to stimulate the activity of the yeast. Pasteur's investigations on fermentation have convincingly proved—at least to most minds—the intenability of a *peculiar motion* and also of *spontaneous generation* as being the true cause of these interesting phenomena.

In addition to a continued life without free oxygen which the yeast cell manifests, there is another feature —exceptional in character—which it possesses, and that is, that its growth is not commensurate with the sugar decomposed. For a given weight of yeast formed one may have ten, twenty, or one hundred times its weight of sugar decomposed. It may be of interest to quote here two experiments—out of many—bearing on this question and also on the influence of dissolved oxygen in a saccharine liquid. M. Pasteur took two flasks partially filled with a liquid containing in each case 150 grammes of sugar; the one (*a*) was almost completely deprived of air, the other (*b*) contained air. A mere trace of yeast (species, *Saccharomyces pastorianus*) was added to each. The fermentation in *a* was sluggish, and after nineteen days, when the experiment was closed, there was still a slight evolution of carbonic acid gas; in *b* the fermentation was active, and ceased entirely on the ninth day. The weight of yeast formed was determined in both cases, as also that of the sugar left undecomposed; these were the results:—

a.	Weight of yeast formed	1·368 grammes.
	Sugar undecomposed	4·600 ,,
b.	Weight of yeast formed ...	1·970 ,,
	Sugar undecomposed	*nil.*

In *b*, therefore, though the liquid was far from being saturated with air, the decomposition of the sugar was completed in eight days, whereas in *a*, even at the end of nineteen days, there was still some sugar left. The weight of yeast formed was as 1 to 76 of sugar decomposed in *b*, and only as 1 to 89 in *a*. In another experiment on a sugar solution completely deprived of air, the whole of the sugar was not decomposed even at the end of three months, the yeast produced was deformed in appearance, and the ratio of yeast to alcohol produced was as 1 to 176. Reversing the experiment so as to obtain a thorough and constant saturation of the liquid with air, the ratio of yeast formed to the alcohol was as high as 1 to 4. In this latter case the yeast acted more as a mycoderm or as a mould than as a true ferment. We may sum up the bearing of the facts elucidated by Pasteur, by the statement that, within given limits defined in Pasteur's experiments, *the production of yeast is directly, and that of alcohol inversely, as the consumption of free oxygen.*

M. Pasteur having demonstrated that the alterations in the yeast, in the wort, and in the beer, are due to the action of microscopic organisms other than the true yeast cells, and that these ferments of disease are killed at a high temperature, it follows that if a boiling wort be cooled under conditions which preclude the entrance of these germs, and if fermented with pure yeast, a beer will be obtained which, so long as not exposed to germ-laden air, must remain unchanged for an indefinite time. M. Pasteur has conferred an additional favour on the practical man by designing an apparatus by which these conditions may be secured, and has carried on his process of fermentation on a large experimental scale in several French breweries with much success so far as flavour and soundness of product are concerned. The general adoption of new and costly appliances must necessarily be slow. Nor is it essential that his suggestions should be in all cases carried out in their entirety. The intelligent brewer who thoroughly masters the key to sound and unsound fermentation will be enabled to secure the one and avoid the other without replacing costly appliances by others vastly more so. To do this, however, the microscope must be in daily use in order to cultivate a purer and purer yeast crop, and his technical processes of skimming and cleansing must be more and more directed by this invaluable instrument. The selection and preservation of yeast, the cultivation of it and the gradual elimination of diseased ferments will henceforth be the object of well-guided efforts, and not of mere chance, as heretofore. The technical reader will do well to read and re-read the work before us; as he becomes master of its method of investigation and of the results obtained, so will he master many of the difficulties of his art.

Some suggestions are given (p. 224, *et seq.*) on the purification of yeast of interest to the brewer as well as to the experimentalist. It would lengthen this notice too much to give the details of the process; it will suffice here to state that the author recommends the use of a weak solution of sugar rendered slightly acid with tartaric acid. In some cases he employs in addition a trace of carbolic acid. Such a liquid destroys some true yeast; it is, however, far more injurious to the ferments of disease. It should not be forgotten that aeration, while injurious to most ferments of disease (mycoderma vini and aceti, of course, excepted), is a great stimulant to the Saccharomyces; hence this method may be adopted either alone or in conjunction with others suggested by him. Those interested in this matter, and especially the brewer, will do well to study carefully the work itself, they will find on this subject, as well as on many others, not alluded to here, much invaluable and suggestive information.

In concluding our brief examination of Pasteur's biological researches on fermentation, a few words on the products formed will not be out of place.

Numerous attempts have from time to time been made to express by a chemical equation the decomposition of glucose sugar by the yeast cell. Until the discovery of succinic acid by Schmidt, and of glycerine by Pasteur, the attempts made were far from being satisfactory. According to Pasteur 100 parts of cane-sugar corresponding to 105·26 parts of grape-sugar give when fermented—

Alcohol	51·11
Carbonic acid	49·42
Succinic acid	0·67
Glycerin	3·06
Matters united with ferments	1·00
	105·26

Therefore ninety-five parts of cane-sugar are broken up into carbonic acid and alcohol, one part is assimilated by the yeast, and four parts are converted into succinic acid and glycerin.

Monoyer has proposed the following equation to represent the decomposition of these four parts of sugar, *e.g.*,

$$\underset{\text{Hydrated cane-sugar.}}{4(C_{12}H_{24}O_{12})} + \underset{\text{Water.}}{6H_2O} = \underset{\text{Succinic acid.}}{2(C_4H_6O_4)} + \underset{\text{Glycerin.}}{12(C_3H_8O_3)}$$
$$+ \underset{\text{Carbonic acid.}}{4CO_2} + O_2,$$

the liberated oxygen supporting the life of the plant. It is impossible at present, however, to formulate the reactions which occur, because they vary according to the temperature, pressure, species of yeast employed, and to the nature of the saccharine liquid. M. Pasteur himself has pointed out that the proportions of succinic acid and glycerin vary in different experiments.

Mr. Horace Brown in some admirable and suggestive experiments on the influence of pressure on the products of fermentation (*Journ. Chem. Soc.*, 1872, p. 570), has shown that in alcoholic fermentation in addition to carbonic acid there is always formed a small quantity of nitrogen, hydrogen, and a hydrocarbon of the marsh gas series; the nitrogen being evolved from the albuminous matter of the wort. By diminishing the pressure the amount of evolved hydrogen increased, and with this an increase in the amount of acetic acid and aldehyde. These products, though very small compared with the alcohol and carbonic acid, the chief resultants of ferment action are yet sufficient to account for the etherial odour of a fermenting tun. Pasteur had previously noticed the production of minute quantities of volatile acids. On electrolysing a weak aqueous solution of invert sugar, Brown obtained carbonic acid, hydrogen, and oxygen, and at the same time an appreciable quantity of aldehyde and acetic acid, together with a small quantity of formic acid. It may be that water is decomposed in fermentation in small quantities precisely as occurs in ordinary vegetation; though highly probable, we have, however, no definite facts in support of the assumption. Our present knowledge of the chemistry of fermentation is somewhat vague and general, and much remains to be done before we shall be enabled by purely physical means to decompose sugar so as to produce the results brought about by the yeast cell.

As we progress in knowledge, so does our power to imitate the products of life-action increase, and assuredly the time will arrive when alcohol will be produced by simple physical or chemical means. For many a year to come, however, we must continue to depend upon the wonderful organisms known as yeast. Their life history and action on liquids have been elucidated by the genius and patient toil of Pasteur, and he has enabled us to select such ferments as are required to produce the result desired, and hence we are no longer the sport of chance.

The brewer and wine-maker are not alone in the debt due to the illustrious Frenchman whose work we have briefly examined; we are all interested in the far wider field of germ action opened out by him, whereby many a disease of man hitherto as dark and unexplained as was fermentation, will be brought under the illuminating light of his teaching.

He who has thus shown us the key whereby we may open the locked-up secrets of nature may rest assured of the gratitude of his fellow-men, given with the greater earnestness and respectful sympathy from the knowledge that our guide has impaired health and sight in his labours for others.

France has given many a great name to the roll of fame, but none more noble or more worthily inscribed thereon than that of Pasteur. CHARLES GRAHAM

CAYLEY'S ELLIPTIC FUNCTIONS

Elementary Treatise on Elliptic Functions. By Arthur Cayley. (Cambridge: Deighton, Bell, and Co.).

THIS is a book thoroughly worthy of the great name of its author. It is difficult to know which to admire most, the grasp of the subject, the extreme simplicity of its exposition, or the neatness of its notation. It will, we think, at once take its proper place as the leading text-book on the subject.

In regard to notation, it seems to us to be thoroughly good throughout, not only in respect of the adoption of Gudermann's suggestion of the very short forms sn, cn, dn, for the sine, cosine, and elliptic radical of the amplitude of the function of the first kind, but throughout. In particular, we note an important typographical simplification in the suppression of the common denominator in long series of fractional formulæ, the denominator being given once for all, and its existence in each separate formula merely indicated by the sign of division (÷) This is a simplification, some equivalent of which we ourselves, and probably most of those who have worked at elliptic functions, have used in our private papers; but it is a new thing, and a very good thing, to see it introduced in a systematic form in a printed book.

Another very useful feature belonging to mechanical arrangement is, that the first chapter contains a general outline of the whole theory, so that its perusal enables the reader to see at a glance the plan and intention of the work. He is thus enabled at once to bring intelligent attention to bear upon his reading, instead of being distracted by the wonder as to what it is all driving at.

The intention of the work is, firstly, the direct discussion and comparison of the three forms of elliptic integral, and of the doubly periodic functions which are regarded as the direct quantities of which these integrals are inverse functions. Then the auxiliary functions Z, H, and H are taken up in an elementary form, and after this the transformation of the elliptic functions, by division or multiplication of the primary integral, with the corresponding change of modulus and amplitude. These are very fully and clearly discussed. In particular, the connection between the transformation of the radical in the elliptic integrals, and the formulæ of multiplication, is clearly brought out. Legendre had left this as a very puzzling, although necessary, inference, which he scarcely stopped to discuss. After this comes a discussion of the *q* functions, with a further discussion of the functions H and H, and then some miscellaneous developments.

The work is strictly confined to elliptic functions and their auxiliaries. The more general theories of Abel and Boole find no place in it, nor is there any general discussion of single and double periodicity such as forms the foundation of the work of Messrs. Briot and Bouquet. There are but few examples of the computation of particular values of the elliptic functions, and no account of general methods of computation, either of isolated values, or of tables, or of the arithmetic connected with them; nor are ultra-elliptic functions touched upon. The geometrical applications or illustrations of the elliptic integrals and functions are but meagre, and no mechanical applications are given.

The arithmetical work is quite rightly omitted. That will find a much better place in the hand-book or introduction which will doubtless accompany or follow the great tables of elliptic functions now being printed for the British Association. There is, however, one point which we think it an omission to notice, and that is the solution of the addition equation by means of auxiliary angles. (See Legendre, "Traité des Fonctions Ell.," vol. i., p. 22; or Verhulst, § 19, p. 40.) It is no defect, again, that the mechanical applications are omitted. These are better studied as they arise, as a part of mechanics rather than of analysis. But as regards geometry, we think there has been done either too much or too little. For instance, we have the usual theory of the representation of the arcs of the ellipse and hyperbola